REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
26-04-2010	Final Report	17 April 2009 - 27-Apr-10

4. TITLE AND SUBTITLE

MALDI TOF Imaging of Latent Fingerprints a Novel Biosignature Tool.

5a. CONTRACT NUMBER
FA8655-09-1-3084

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Michael Gozin

5d. PROJECT NUMBER

5d. TASK NUMBER

5e. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Tel Aviv University
Tel Aviv University
Tel Aviv 69978
Israel

8. PERFORMING ORGANIZATION REPORT NUMBER
N/A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

EOARD
Unit 4515 BOX 14
APO AE 09421

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
Grant 09-3084

12. DISTRIBUTION/AVAILABILITY STATEMENT: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT: This project is aimed to develop novel methodology for latent fingerprints visualization with simultaneous chemical analysis based on MALDI-MSI. The overall objectives of our research program are: (i)Studying of a sweat chemical composition (mostly focusing on compounds with molecular weight larger than 3,000 Da) of a group of individuals (30-40 adults, preferably 50% men and 50% women) by HPLC-electrospray Q-TOF and MALDI TOF-TOF mass spectrometry techniques. To assess possibility of fingerprints visualization with simultaneous chemical analysis based on MALDI-MS, model experiment was performed. Specifically, finger of thirty-year-old man have been lightly coated with ointment containing tocopherol and imprinted on stainless-steal MALDI plate. Application of low-concentrated tocopherol allows efficient laser ionization without use of matrixes or additional treatment of the fingerprint. The result of the MS imaging scan clearly shows that MALDI-MSI is very promising analytical tool to be used in biometric research with good enough resolution and sensitivity, even for fast-mode of scan (100 distance step resolution) and no advanced image processes approaches, which can greatly improve raw-data processing in terms of resolution and contrast. Interestingly, MS method optimized for molecular peak and main fragments of tocopherol (395 m/z) gave signal increase of over one order of magnitude. However, no clear fingerprint pattern was achieved for fingerprints without additives. The main reason to this is a lack of efficient method for ionization, spectrometry and data-processing of sweat components at this point. To develop improved mass spectrometry methods optimized for fingerprint scanning and signals enhancing, better understanding of sweat components mass spectrometry was required. As a result, the major objective of the second phase of this research was characterization of relevant for biometry sweat components and MALDI-MS methods optimization. The most intensive, significant and informative signal for fingerprint biometry applications can be attributed to 1.2 to 10.0 Kd mass range, which characteristic for peptides and small proteins. Series of matrixes and deposition techniques was evaluated for fingerprint signal enhancing in this mass range. (such as: 2,5-dihydroxybenzoic acid (DHB), 2-(4-hydroxy-phenylazo)benzoic acid (HABA), -cyano-4-hydroxy-cinnamic acid (CHCA) and sinapinic acid was applied using following techniques: dried-droplet, vacuum-drying, fast-evaporation, sandwich and wet/dry spraying). The best found methodology included use of CHCA matrix and a combination of spray and drop-casting deposition techniques. This procedure allowed MALDI-MS analysis of fingerprint deposited directly on stainless steel plate. In conclusion, this project is aimed to develop novel methodology for latent fingerprints visualization with simultaneous chemical analysis based on MALDI-MSI. Here we demonstrated the possibility of latent fingerprint visualization with suitable matrix by MALDI-MSI. Moreover, possibility of individual peptide identification in a fingerprint was shown. These findings were supported by RP HPLC and PAGE analyses.

15. SUBJECT TERMS
EOARD, Biotechnology, Biochemistry

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18, NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			TAMMY SAVOIE, Lt Col, USAF
UNCLAS	UNCLAS	UNCLAS	UL	10	19b. TELEPHONE NUMBER *(Include area code)* +44 (0)1895 616459

First Year Annual Report – Grant # 093084

23 April 2010

By: Mr. Bogdan Belgorodsky, Dr. Ludmila Fadeev, Dr. Michael Gozin
School of Chemistry, Tel Aviv University, Tel Aviv 69978, Israel.

Introduction

Since the first conviction using fingerprints as evidence in 1902, the fingerprint analysis at crime scenes has become a key approach in the identification and apprehension of individuals for forensic purposes. They provide personal identification, which among few biometric signatures is considered as truly unique and invariable for each individual. More recently, increasing identity fraud has created a growing need for biometric technology for positive person identification in a number of non-forensic applications, such as a driver's license, welfare disbursement, access control, and credit card identification. Because biometric identifiers cannot be easily misplaced, forged or shared, they are considered more reliable for personal identification than traditional token- or knowledge-based methods.

From fingertips to wrist, the palm of a hand is covered by minute ridges of skin, called papillary or friction ridges, with the depressions between the ridges termed furrows. The ridges and furrows curve, especially on the fingertips, to form complex patterns. Indeed, every fingertip has a unique and distinctive ridge pattern, with no two fingers ever having been found with identical characteristics.

The friction ridges have pores at intervals along their length. A pore is the opening on the skin surface of a sweat gland duct. Sweat or eccrine glands are found on almost all skin surface areas of the body, with a high concentration on the palms of the hand (approximately four per square millimeter), so the ridges are coated with a thin film of perspiration. According to the present knowledge, eccrine glands' sweat consists 98% of water and 2% dry materials. Dry material consists 45% metal salts and a small amount of a highly complex mixture of organic compounds (including lactate, urea, amino acids and various peptides and proteins). However, the organic content of perspiration on the ridges can be increased by sebum, the oils and fatty substances secreted by the sebaceous glands largely associated with hair follicles. Finger contact with the face, neck or scalp therefore increases the sebum content of the perspiration on the fingerprint ridges. Hence, touching a surface with a finger leaves contours of fingerprint residue, ranging from ordinary eccrine sweat to a sebum-rich deposit, which is primarily comprised of fatty acids, glycerides, cholesterol, squalene and variety of lipid esters.

For years, forensic scientists have been seeking new methods or trying to improve existing techniques for the visualization of latent fingerprints. A wide range of physical and chemical techniques and instrumentation associated with surface chemistry imaging and characterization has improved remarkably over recent years. The structured combination of optical methods (diffused reflection, luminescence, UV absorption and reflection), physical methods (powdering, vacuum metal deposition (VMD), small particle reagent), physico-chemical methods (physical developer, multi-metal-deposition (MMD), iodine, cyanoacrylate) and chemical methods (ninhydrin and its analogues, DFO,

etc.), permits a rational and highly efficient processing of the secretions deposited by the fingers on a great variety of substrates. To visualize these developed fingerprints, alternative light sources or laser-based detection methods are commonly used. In addition, the optical readout of latent fingerprints, which required data interpretation, and increase of computation power resulted in advanced numerical algorithms for data collection and processing. These conventional techniques are quite effective in the recovery of latent fingerprints under many ordinary circumstances. However, like all other existing techniques, these optical methods do not work in all possible cases and certain types of latent fingerprints or object surfaces with unique characteristics: surfaces with multicolored backgrounds, surfaces contaminated with blood or other body fluids, and other porous or nonabsorbent surfaces may be problematic. The major challenges in fingerprint detection with laser techniques stem from both low emission levels from the fingerprint secretions and interferences from the background signal, for example the background fluorescence when imaging fingerprints on the surface of banknotes.

Mass spectrometry is one of the major techniques for analysis and characterization of various organic and inorganic substances with unmatched sensitivity (at levels as low as attomols). Mass spectrometry is also rapidly becoming an analytical tool of choice to probe directly the molecular composition of very complex biological samples, such as tissue sections. Over recent years, the determination of cholesterol, squalene, fatty acid esters extracted from fingerprint by GC Mass Spectroscopy was demonstrated. MS analysis includes a broad array of bio-molecules, such as peptides, proteins, polysaccharides, and polynucleotides etc. Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) is novel type of visualization technique in which UV pulsed laser is rastering sequentially over a defined area of a sample while acquiring a mass spectrum from every location. From this array of spectra, analyte-specific images (molecular profiles) can be generated based on the selected masses, mapping precisely these analytes location on a surface.

Results

MALDI-MSI Analysis of Fingerprints

To assess possibility of fingerprints visualization with simultaneous chemical analysis based on MALDI-MS, model experiment was performed. Specifically, finger of thirty-year-old man have been lightly coated with ointment containing tocopherol and imprinted on stainless-steal MALDI plate. Application of low-concentrated tocopherol allows efficient laser ionization without use of matrixes or additional treatment of the fingerprint. The result of the MS imaging scan presented in Figure 1 and clearly shows that MALDI-MSI is very promising analytical tool to be used in biometric research with good enough resolution and sensitivity, even for fast-mode of scan (100 μ distance step resolution) and no advanced image processes approaches, which can greatly improve raw-data processing in terms of resolution and contrast.

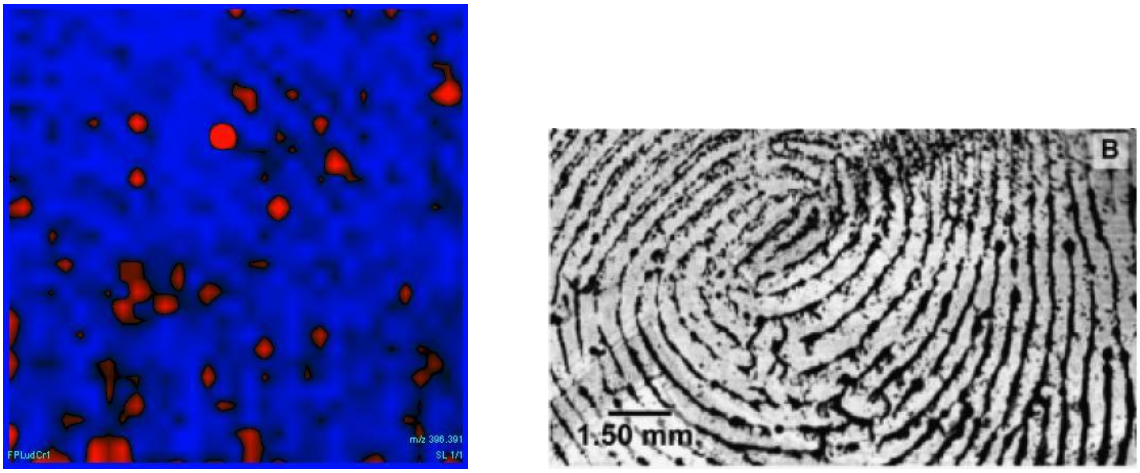


Figure 1. A) MALDI-TOF MS image of fingerprint with tocopherol. B) Sample of infrared image obtained by plotting the band intensity at 1016 cm^{-1}; N.J. Crane et al. *J. Forens. Sci*. **2007.**

Interestingly, MS method optimized for molecular peak and main fragments of tocopherol (395 m/z) gave signal increase of over one order of magnitude (Figure 2A,B). However, no clear fingerprint pattern was achieved for fingerprints without additives. The main reason to this is a lack of efficient method for ionization, spectrometry and data-processing of sweat components at this point. To develop improved mass spectrometry methods optimized for fingerprint scanning and signals enhancing, better understanding of sweat components mass spectrometry was required.

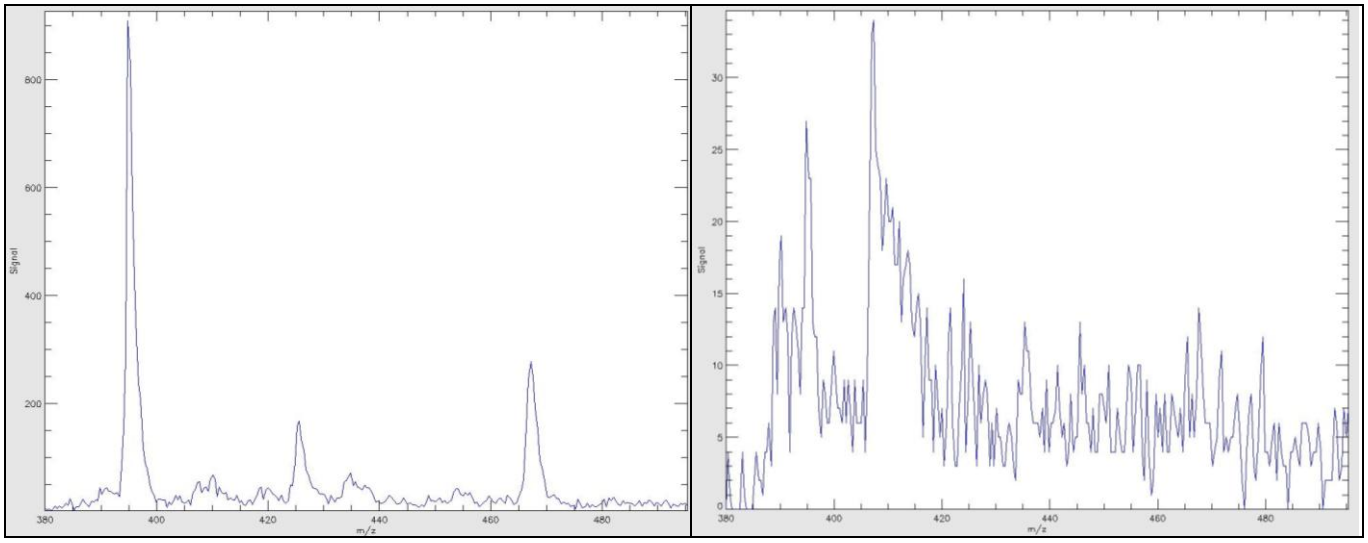


Figure 2. Representative mass spectra of tocopherol-enhanced fingerprint in point with *left*) high intensity signal (Signal of ~700 arbitrary units); *right)* low intensity signal – background (~30 arbitrary units).

As a result, the major objective of the second phase of this research was characterization of relevant for biometry sweat components and MALDI-MS methods optimization. The most intensive, significant and informative signal for fingerprint biometry applications can be attributed to 1.2 to 10.0

KDa mass range, which characteristic for peptides and small proteins. Series of matrixes and deposition techniques was evaluated for fingerprint signal enhancing in this mass range. (such as: 2,5-dihydroxybenzoic acid (DHB), 2-(4-hydroxy-phenylazo)benzoic acid (HABA), α-cyano-4-hydroxy-cinnamic acid (CHCA) and sinapinic acid was applied using following techniques: "dried-droplet", "vacuum-drying", "fast-evaporation", "sandwich" and wet/dry spraying). The best found methodology included use of CHCA matrix and a combination of spray and drop-casting deposition techniques. This procedure allowed MALDI-MS analysis of fingerprint deposited directly on stainless steel plate.

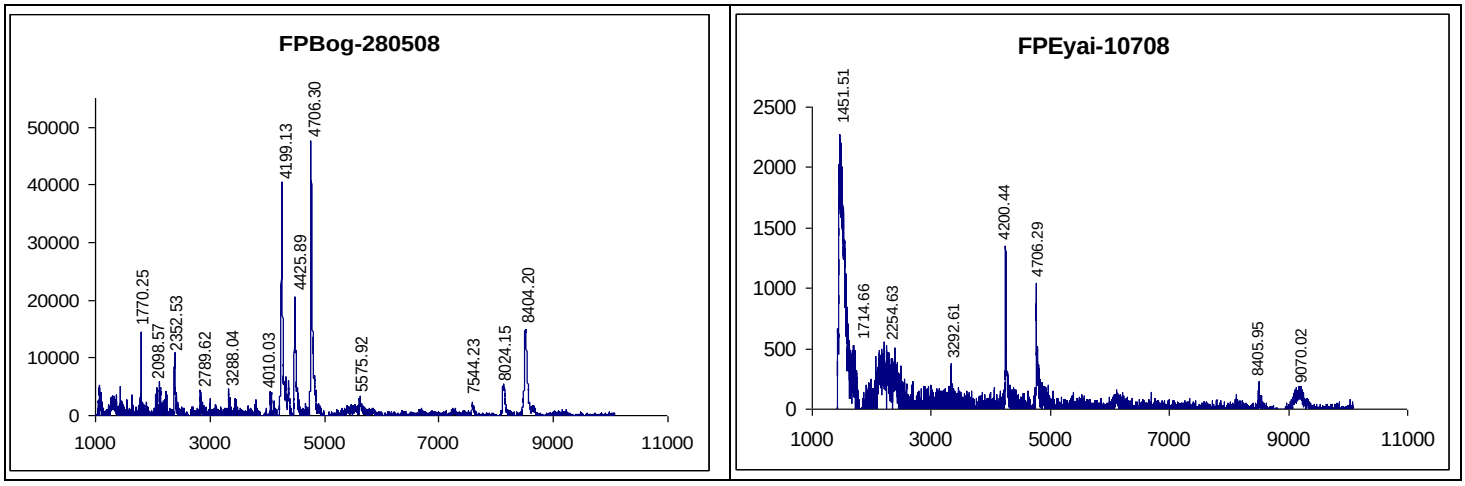


Figure 3. Fingerprint mass spectra of male subjects *(left*) FPBog and *(right)* FPEyal.

Interesting tendencies can be found even for small statistic distribution that was studied in this case. Specifically, mass spectra of 2 men and 2 women fingerprint showed both common and individual peaks patterns (Figures 3 and 4). Presence of well-known antimicrobial peptide dermicidin DCD-1 (4706.3 Da) was clearly detected for all subjects. Additional common to all samples peptides was found at 2254-2353, 4200-4706 and 8400 Da. However, variability in intensity of these signals was observed for different subjects as a result of individual distribution abundance. Moreover, presence of unique for each subject peptides was detected in the spectrum, such as 1770.25 and 4425.89 Da in Figure 3A, 9070.02 Da in Figure 3B, 5252.09 Da in Figure 4A and 2159.94 and 4010.76 in Figure 4B. These results show possibility of person identification by MALDI TOF-TOF mass spectrometry techniques.

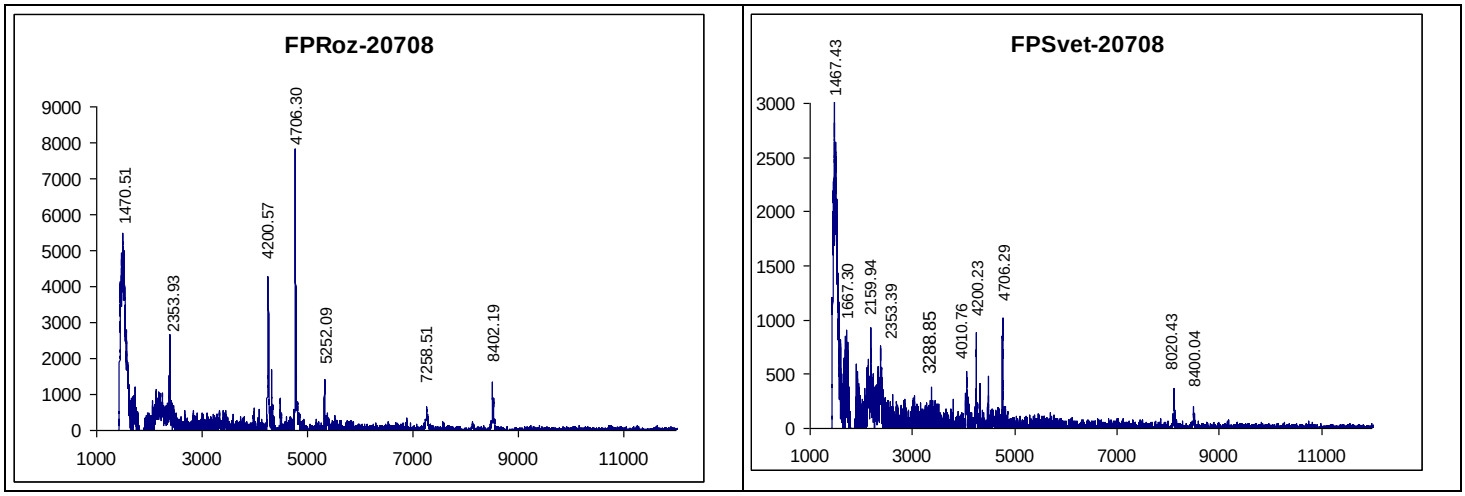


Figure 4. Fingerprint mass spectra of female subjects *left*) FPRoz and *right)* FPSvet.

In the next series of experiments was aimed to characterize previously described sweat compounds. For that purpose previously described sweat collection procedure was optimized. Collected secretions were pre-concentrated and gel-filtrated through Sephadex G-10 Column to remove small molecules (MW < 1000 Da).

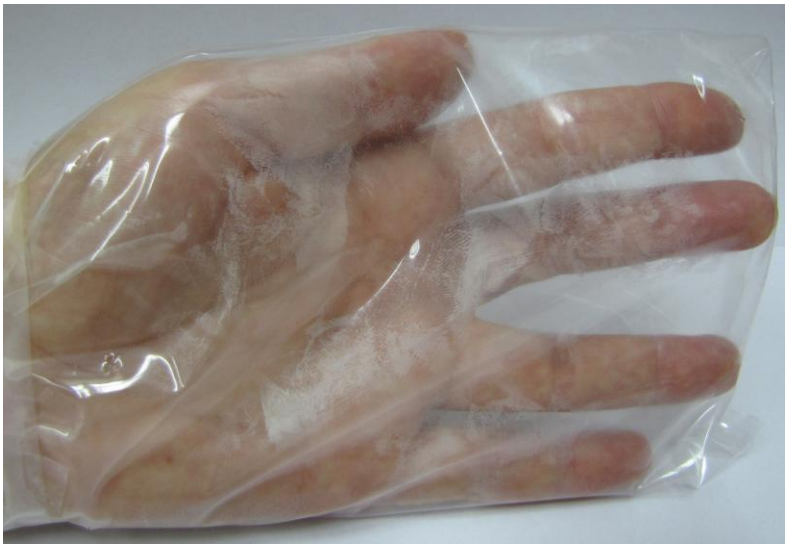


Figure 5. Sweat collection procedure.

MALDI-MS analysis of the concentrated sweat samples demonstrated features similar to the mass spectrum of the subjects fingerprint (Figure 6). However, as it can be expected some differences and additional peaks were found due to more eccrine nature of the collected secretions.

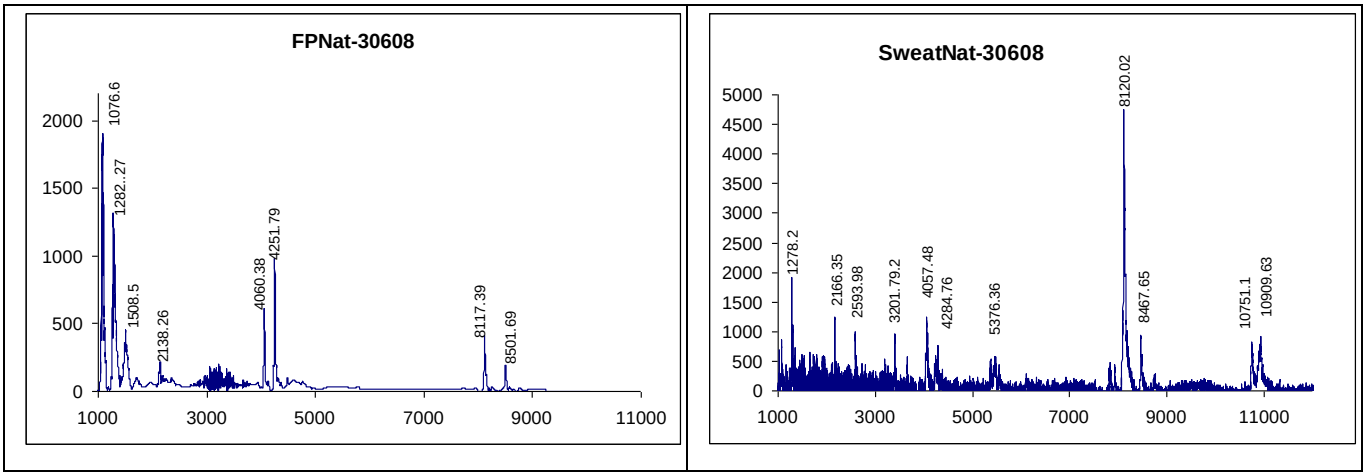


Figure 6. Mass spectra of female subjects Nat *(left*) fingerprint and *(right)* sweat.

Additional analytical approaches were applied for characterization of the found in sweat entitles. Namely, RP-HPLC analyses show the separation of 12-18 peptides in pure hand sweat (Figures 7). Few series of peaks with similar retention times (RT), quantity ratios and resembling spectra are observed in all the chromatograms.

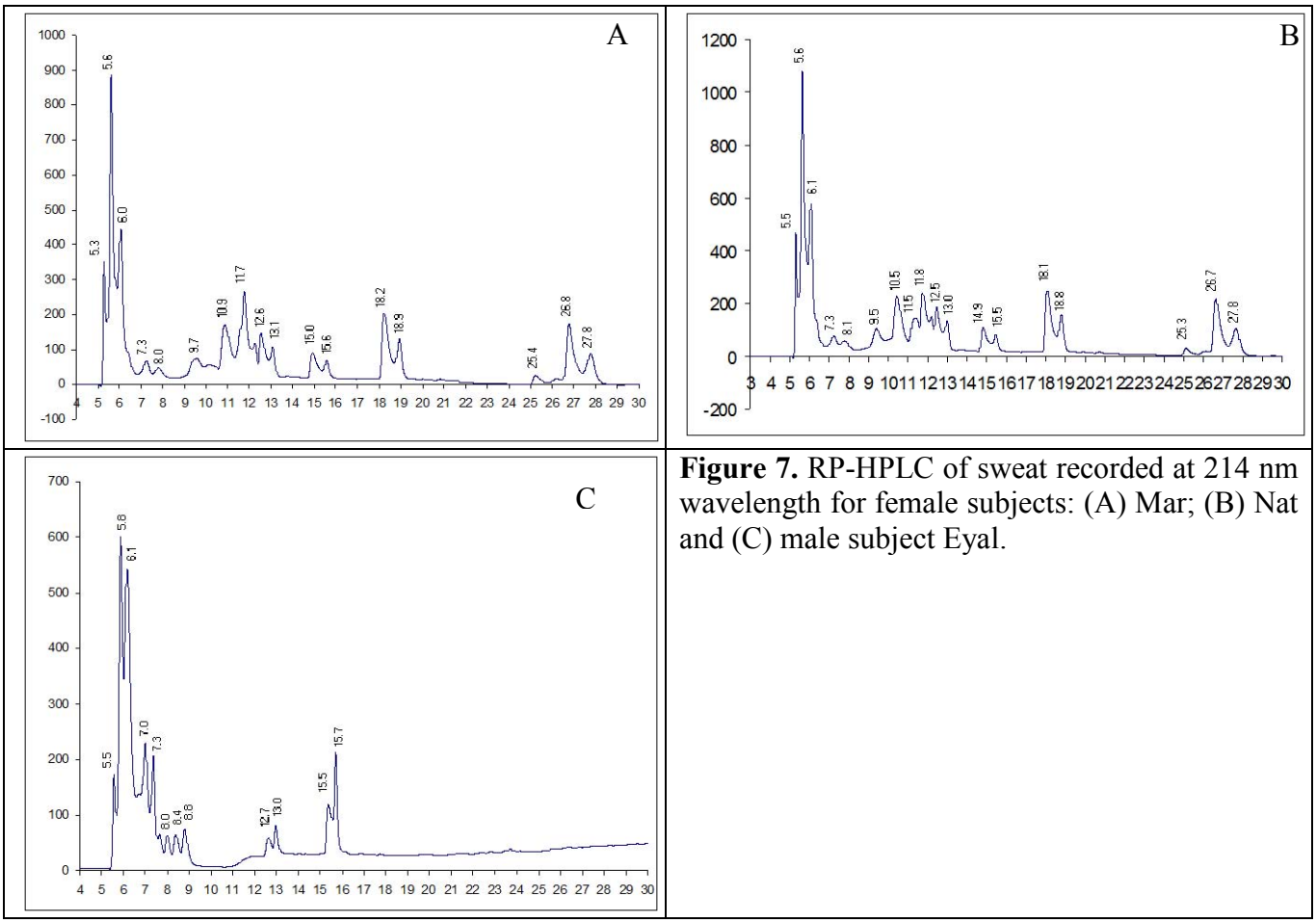
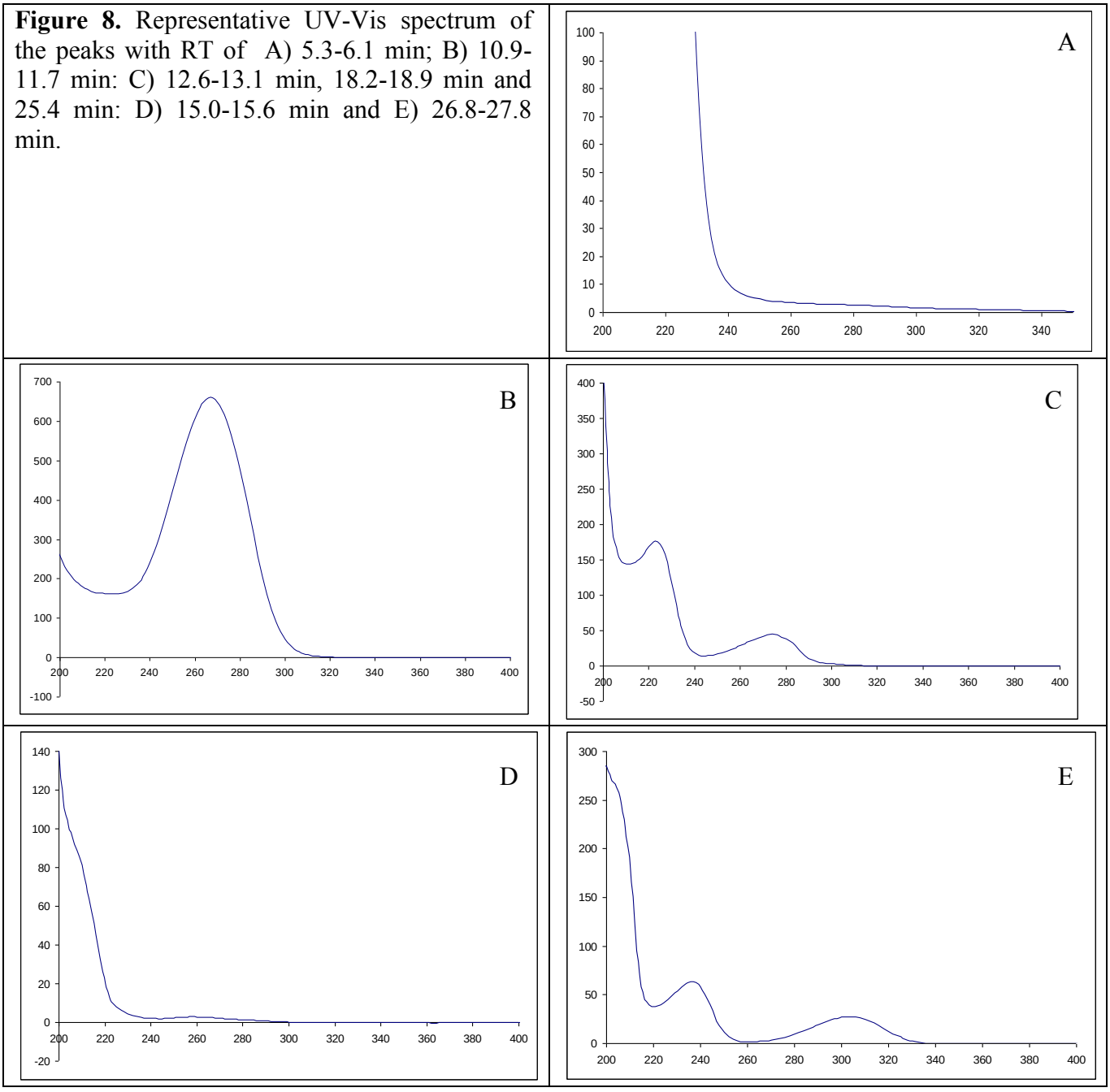


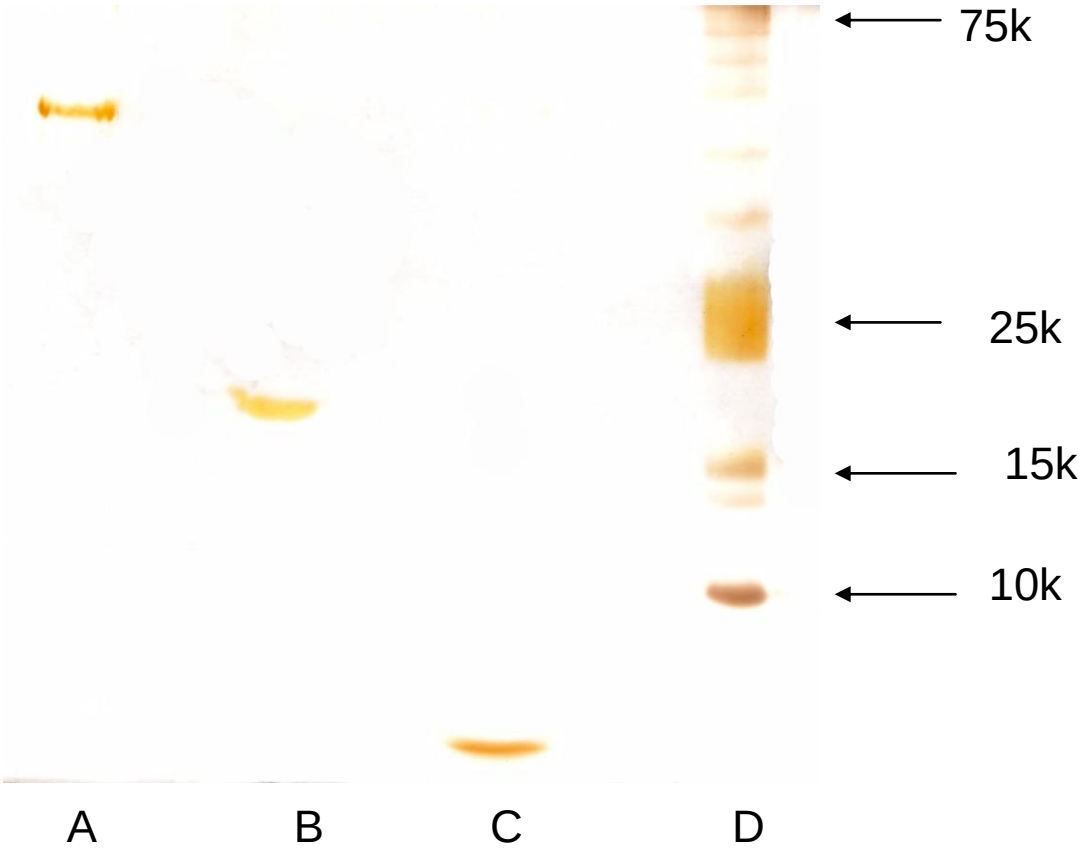
Figure 7. RP-HPLC of sweat recorded at 214 nm wavelength for female subjects: (A) Mar; (B) Nat and (C) male subject Eyal.

The first series consist of 3 peaks characterized by the retention times of 5.3, 5.6 and 6.1 min. The spectra of these peaks are showing spectrum characteristic to highly hydrophilic peptides with low content of aromatic amino acids (Figure 8A). Next series of peaks eluted with RT of 10.9 and 11.7 min demonstrating more hydrophobic behavior and absorbance at 260 nm (Figure 8B). Peptides eluted at the RT of 12.6-13.1 min, 18.2-18.9 min and 25.4 min have more hydrophobic nature which is consistent with longer peptides containing aromatic side-chains and characteristic absorption (Figure 8C) at 214 nm (peptide bonds) and 270 nm (aromatic side chains). Whereas, peptides eluted at the RT of 15.0 and 15.6 min are also long, but has relatively low content of aromatic amino acids (Figure 8D). The last series of peaks with RT of 26.8 and 27.8 min can be assigned to large, hydrophobic peptides with incorporation of unknown metabolites having absorption at 306 nm (Figure 8E).

Figure 8. Representative UV-Vis spectrum of the peaks with RT of A) 5.3-6.1 min; B) 10.9-11.7 min: C) 12.6-13.1 min, 18.2-18.9 min and 25.4 min: D) 15.0-15.6 min and E) 26.8-27.8 min.



Alternative technique was applied to verify our previous conclusions. Specifically, female subjects hand sweat was separated by SDS-PAGE (17%). Bovine serum albumin and β-lactoglobulin were used as reference and loading control. Main component for sweat has band with mass matching <10 KDa marker (Figure 9), which is in good correlation with mass previously found by MALDI-MS for the largest MS peak (8120 Da) in both fingerprint and equine sweat spectra.



A – BSA;
B – lactoglobulin;
C – hand sweat;
D – protein marker.

Figure 8. SDS-PAGE of sweat collected from female subject Nat.

In conclusion, this project is aimed to develop novel methodology for latent fingerprints visualization with simultaneous chemical analysis based on MALDI-MSI. Here we demonstrated the possibility of latent fingerprint visualization with suitable matrix by MALDI-MSI. Moreover, possibility of individual peptide identification in a fingerprint was shown. These findings were supported by RP HPLC and PAGE analyses.

Materials and Methods

Collections and storage of sweat samples. Hands skin of a subject was gently cleansed with 50% ethanol aqueous solution and air dried. Sweat was collected inside a clean plastic pouch over 30-50 min period by DDW wash. 30 collected water sweat samples from one hand was concentrated about 10 time by lyophilizing and were passed through Sephadex G-10 Medium columns in 1mM NaPi, pH 7.2 to remove small (molecules up to 1000 Da) interfering substances such as salts, lipids, glucose, urea, amino acids. Clean sweat was pooled and lyophilized for storage in -4°C.
A weight of peptides was measured on wavelength 595 nm by *Bio-Rad Protein Assay* method relative to quantity of Bovine Serum Albumin . This was 37.8 μg in 200 μl of clean sweat.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis (PAGE) of native (non-denaturated) sweat protein was performed to determine individual differences in sweat protein composition. Acrylamide was cast with a continuous gradient from 7% stacking gel (top) to 17% separation gel (bottom).Thus 17% acrylamide-bis-acrylamide gel contains 0.56 M Tris buffer pH 8.8, 0.1%(w/v) SDS. Trisglycine running buffer contains 3.9 mM Tris-HCl, 0.2 M glycine, 0.001%w/v) SDS. The current used was 40-50 mA in the course of time 180 min. The gel was then fixed and staining by Silver Staining Kit method [1].

Identification of sweat proteins by Reverse phase HPLC. 50μl of clean after G10 column sweat was applied to RP-HPLC on a Protein & Peptide C18 column VYDAC HPLC Columns (250×4.6mm). A linear gradient from 100% solvent A (5% ACN with 0.05% THF in H_2O) to 80% solvent B (80% ACN with 0.05% TFA in H2O) over 35min was used; a flow rate was 0.6 ml/min, pressure 116 Atm. The elution peaks were 214 nm for peptide bonds and 280 nm for aromatic amino acids.